

Intercomparison of Analytical Methods for Arsenic Speciation in Human Urine

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An intercomparison exercise was conducted for the quantification of arsenic species in spiked human urine. The primary objective of the exercise was to determine the variance among laboratories in the analysis of arsenic species such as inorganic As (As^{+3} and As^{+5}), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA). Laboratories that participated had previous experience with arsenic speciation analysis. The results of this interlaboratory comparison are encouraging. There is relatively good agreement on the concentrations of these arsenic species in urine at concentrations that are relevant to research on the metabolism of arsenic in humans and other mammals. Both the accuracy and precision are relatively poor for arsenic concentrations of less than about 5 $\mu\text{g/l}$. **Key words:** arsenate, arsenic, arsenite, dimethylarsinic, intercomparison, monomethylarsonic, speciation, urine. *Environ Health Perspect* 105:650–653 (1997)

An intercomparison exercise was sponsored by the Electric Power Research Institute (EPRI) for the quantification of arsenic species in spiked human urine. The primary objective of the exercise was to determine the variance among laboratories in the analysis of arsenic species such as inorganic As (As^{+3} and As^{+5}), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA). Only laboratories with previous experience with arsenic speciation analysis participated. The results in this report are not identified by specific laboratory.

Materials and Methods

Samples. A urine composite collected from three adults was frozen, thawed, and decanted to reduce the amount of suspended matter and then spiked with the concentrations of arsenic shown in Table 1. The chemicals used to spike the urine included sodium arsenite and sodium arsenate (99.9% pure; Mallinckrodt, Phillipsburg, NJ), methane arsonic acid (no purity given; Ansul Inc., Marinette, WI), and sodium cacodylate (no purity given; Ted Pella, Inc., Redding, CA). The unspiked urine contained approximately 0.4 $\mu\text{g/l}$ inorganic arsenic, 0.5 $\mu\text{g/l}$ MMA, and 2 $\mu\text{g/l}$ DMA.

After the urine was spiked, approximately 200-ml aliquots were frozen in polyethylene bottles and shipped on ice to seven laboratories by air courier. Included in the

shipments were standard solutions of 10 mg/l of MMA and DMA and 5 ml of National Institute of Standards and Technology (NIST; Gaithersburg, MD) standard reference material (SRM) 2670 (toxic metals in human urine), which is certified at $480 \pm 100 \mu\text{g/l}$ total arsenic.

The shipping procedures were designed to keep the urine samples frozen or cold for about 48 hr. The samples were shipped by air courier; however, three laboratories received samples that were not cold (Table 2).

Analytical methods. Several different types of analytical methods were used to speciate the arsenic in the urine samples (Table 3). Four laboratories used hydride-cryogenic trap-atomic absorption spectroscopy (AAS), two used ion exchange, and one used high-performance liquid chromatography (HPLC) to separate inorganic and organic species. Only two laboratories (Numbers 4 and 7) determined As^{+3} and As^{+5} . Because these two laboratories used different methods, a limited comparison can be made for inorganic speciation. The methods for the determination of total arsenic included inductively coupled plasma mass spectrometry (ICP-MS), extraction-graphite furnace atomic absorption (GFAA), and digestion or ashing followed by hydride-AAS (Table 3).

The method detection limits [MDL; defined as the minimum concentration of a

substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte (1)] reported by the participating laboratories are shown in Table 4. Sample volumes used were not reported. The laboratories were able to quantify the arsenic species in all but a few cases in the Sample 1A, the lowest spike sample.

For each sample type and species of arsenic, laboratories were compared using a one-way analysis-of-variance (ANOVA) on the natural logarithm transformed concentration. The transformation was conducted to reduce the inequality of the within-laboratory variances.

Results and Discussion

Each of the seven laboratories analyzed three types of samples: 1) known standard solutions of MMA and DMA; 2) known NIST SRM 2670; and 3) three spiked urine samples of unknown concentration. In most cases, the laboratories analyzed each sample in triplicate.

Laboratories analyzed two known methylated arsenic standard solutions that were distributed with the urine samples. These standards contained 10 ppm MMA and 10 ppm DMA as arsenic. The purpose was to determine whether there was bias among laboratories due to the availability of calibrating standards. The participating laboratories analyzed these two standards for either the methylated form and/or total arsenic. The results (Table 5) indicate that

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Table 1. Spiking concentrations for three urine samples

Sample	Arsenic ($\mu\text{g/l}$)			
	As^{+3}	As^{+5}	MMA	DMA
1A	1.5	1.5	1.5	4.0
2A	4.0	2.0	6.0	15.6
3A	12.5	12.5	22.0	44.0

Abbreviations: MMA, monomethylarsonic acid; DMA, dimethylarsinic acid.

Table 2. Condition of samples after shipment

Laboratory	Days in transit	Condition when received
1	2	Warm
2	NR	Frozen
3	10	Warm
4	8	Warm
5	NR	Cold
6	NR	Frozen
7	NR	Frozen

NR, not reported.

all laboratories agree within $\pm 19\%$ on the nominal concentration of the methylated compounds. The three laboratories that analyzed these two standards for total arsenic were within $\pm 15\%$ of the nominal value, except for one value of $+37\%$.

The results for the analysis of the SRM 2670 for arsenic species are shown in Table 6. This reference material was prepared by NIST by spiking inorganic arsenic into urine. Therefore, more than 80% of the arsenic is in the inorganic form. The mean total arsenic of 509 $\mu\text{g/l}$ reported by the seven laboratories is in good agreement with the certified value of $480 \pm 100 \mu\text{g/l}$. Both MMA and DMA were quantified by most laboratories. Laboratory Number 4 determined the inorganic arsenic to be arsenate (As^{+5}). The sum of mean inorganic, MMA, and DMA was 487 $\mu\text{g/l}$, which is 15 $\mu\text{g/l}$ less than the total arsenic determined by AAS or ICP-MS. This difference between total arsenic and the sum of the arsenic species detected could be due to other arsenic compounds in the urine that were not detected by most of the speciation methods used; methods for total arsenic may not detect all species of arsenic present in a sample. Laboratory Number 4, using HPLC-ICP-MS, reported approximately 30 $\mu\text{g/l}$ of arsenobetaine.

The results from the analysis of the three spiked urine samples by the participating laboratories are shown in Table 7. Each laboratory analyzed three replicates. Total As (Table 7) refers to arsenic analyzed by methods that quantify both inorganic and organic arsenic; however, the effectiveness of some of the total methods used on very stable organic arsenic compounds, such as arsenobetaine, is not known.

Six laboratories reported results for inorganic arsenic, two laboratories reported results for As^{+3} and As^{+5} , seven laboratories reported results for MMA and DMA, and seven laboratories reported results for total As. The mean concentration and coefficient of variation (CV) for the results are shown in Table 7 for each of the three spiked urine samples.

The mean arsenic concentrations for the seven laboratories are compared with the concentrations of arsenic species spiked in Samples 1A, 2A, and 3A (Table 8). The percentage recoveries (the ratio of mean arsenic detected to concentration spiked) in Table 8 range from 33 to 155% in Sample 1A, the sample spiked with the lowest concentration of arsenic species. The percentage recoveries improve significantly for Sample 2A and Sample 3A.

Effects from storage and shipping. Urine samples were shipped frozen by air courier on 8 August 1994. Three laboratories

Table 3. Analytical methods used by each laboratory

Laboratory	Methods for arsenic speciation	Methods for total arsenic
1	Hydride-cryogenic trap-AAS	Wet digestion, hydride-AAS
2	Ion exchange, toluene extraction, GFAA (inorganic) Ion exchange, GFAA (MMA and DMA)	Toluene extraction, GFAA
3	Hydride-cryogenic trap-AAS ^a	Extraction, GFAA ^b
4	HPLC-ICP-MS	ICP-MS
5	Hydride-cryogenic trap-AAS	Ashing with $\text{Mg}(\text{NO}_3)_2$, hydride-AAS
6	Ion exchange, hydride AAS ^c	Dry ashing, hydride-AAS ^d
7	Hydride-cryogenic trap-AAS ^e	ICP-MS

Abbreviations: AAS, atomic absorption spectroscopy; GFAA, graphite furnace atomic absorption; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; ICP-MS, inductively coupled plasma mass spectrometry.

^aFrom Howard and Comber (2).

^bFrom Subramanian (5).

^cFrom Tam et al. (3).

^dFrom Vahter et al. (6).

^eFrom Crecelius et al. (4).

Table 4. Method detection limits in units

Laboratory	Arsenic ^a					Total As
	Inorganic As	As^{+3}	As^{+5}	MMA	DMA	
1	1.5	—	—	2.0	4.0	0.7
2	—	—	—	—	0.8	2.5
3	1.2	—	—	0.8	1.2	3.8
4	0.5	0.5	0.5	0.5	0.5	0.5
5	0.1 ng/l	—	—	0.1 ng/l	3.0 ng/l	1.0 ng/l
6	2.0 ng/l	—	—	2.0 ng/l	2.0 ng/l	2.0 ng/l
7	0.05	0.05	0.05	0.1	0.1	3.0

Abbreviations: MMA, monomethylarsonic acid; DMA, dimethylarsinic acid.

^aGiven as $\mu\text{g/liter}$ except where ng/l is indicated.

Table 5. Concentrations of MMA and DMA determined in 10 ppm standard solutions as methylated arsenic compound or total arsenic

Laboratory	Arsenic (mg/l)			
	MMA standard (10 ppm)		DMA standard (10 ppm)	
	MMA	Total As	DMA	Total As
1	10.1	—	9.7	—
2	—	—	11.9	9.9
3	9.2	13.7	10.4	8.5
4	—	9.23	—	10.4
5	9.6	—	10.3	—
6	8.5	—	9.1	—
7	11.2	9.7	9.5	10.7

Abbreviations: MMA, monomethylarsonic acid; DMA, dimethylarsinic acid.

Table 6. Concentrations of arsenic species and total arsenic in NIST SRM 2670 urine sample (certified value $480 \pm 100 \mu\text{g/l}$ total arsenic)

		Arsenic (µg/l)						
Sample	Laboratory	Inorganic			MMA	DMA	Sum As ^b	Total As ^c
		Total inorganic As ^a	As ⁺³	As ⁺⁵				
SRM 2670	1	476	—	—	22.7	—	—	495
	2	—	—	—	—	54.8	—	432
	3	417	—	—	9.3	44.0	471	570
	4	362	<1	362	16.7	60.0	439	475
	5	431	—	—	15.1	69.9	516	—
	6	385	—	—	14.7	38.7	438	486
	7	509	—	—	14.5	45.5	569	556
Mean		430	—	—	15.5	52.2	487	502
CV		13%	—	—	28%	22%	11%	10%

Abbreviations: MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; SRM, standard reference material; CV, coefficient of variation.

^aTotal inorganic As includes As^{+3} and As^{+5} .

^bSum of inorganic arsenic, MMA, and DMA.

^cTotal As determined by either atomic absorption or inductively coupled plasma-mass spectrometry.

received samples that were at room temperature and that had been in transit for 2–10 days. All samples were analyzed during August and September 1994. There is no indication that the shipping procedures affected the arsenic speciation. The results for the laboratories that received warm samples are similar to those from laboratories that received cold or frozen samples.

Differences among analytical methods.

There does not appear to be a systematic difference among analytical methods for arsenic speciation based on this exercise using four arsenic species spiked at between 1.5 and 44 µg/l. Four laboratories (Numbers 1, 3, 5, and 7) used hydride-cryogenic trap-AAS. Laboratory Number 2, which used ion exchange-GFAA, reported similar results for

DMA. Laboratory Number 6, which used ion exchange-hydride-AAS, reported concentrations similar to the mean for all laboratories. Laboratory Number 4, which used HPLC-ICP-MS, also reported results similar to the mean.

There were no trends in statistical differences found among laboratories based on the ANOVA; that is, no laboratory stood out in the analysis. Statistical differences ($p < 0.05$) were found when the within-laboratory variances were small compared with the between-laboratory variance. Laboratory mean concentrations, either high or low, were not consistent across sample type or arsenic species.

Laboratories (Number 4 and Number 7) reported results for As^{+3} and As^{+5} in spiked samples 2A and 3A, which had been spiked with detectable levels of these species. The As^{+3} and As^{+5} results for Sample 3A were similar for these two laboratories, and the mean percentage recovered ranged from 87% for As^{+3} to 121% for As^{+5} , indicating that some of the As^{+3} may have oxidized to As^{+5} during storage or analysis (Table 8). Results for Sample 2A had mean As^{+3} and As^{+5} recoveries of 70% to 60%, respectively. There is no indication that the oxidation of As^{+3} to As^{+5} is a serious problem for these methods. Research reported by Hughes et al. (7) demonstrated that on-column oxidation of As^{+3} to As^{+5} can occur under certain pH conditions using some HPLC methods. However, they also reported that an ion-pairing HPLC method was developed for analysis of As^{+3} , which eliminated the oxidation of As^{+3} .

An investigation of the stability of arsenite was conducted by Laboratory Number 4, which used HPLC for speciation. They found that at the pH values of 6.0 and 3.75 in the mobile phase no on-column oxidation occurred. At values above pH 10, some oxidation was observed after 1 hr, and at pH 13, half the arsenite was oxidized to arsenate during the analysis.

Conclusions

The results of this interlaboratory comparison are encouraging. There is relatively good agreement on the concentrations of three arsenic species (inorganic arsenic, MMA, and DMA) in urine at concentrations that are relevant to research on the metabolism of arsenic in humans and other mammals. Both the accuracy and precision are relatively poor for arsenic concentrations of less than about 5 µg/l because the method detection limits for some of the laboratories are in the range of 1–5 µg/l. Because only two laboratories reported results for arsenite and arsenate, additional intercomparisons are needed to evaluate comparability of laboratories for these species.

Table 7. Concentrations of arsenic species and total arsenic in urine samples

		Arsenic (µg/l)						
Sample	Laboratory	Inorganic			MMA	DMA	Sum As ^b	Total As ^c
		Total inorganic As ^a	As ⁺³	As ⁺⁵				
1A	1	2.7	—	—	1.7 ^d	8.3	12.7	15.1
	2	—	—	—	—	11.2	—	9.1
	3	2.5	—	—	1.4	7.6	11.4	9.9
	4	1.6	1.6	<1.0	2.7	9.5	13.8	24.1
	5	1.3	—	—	1.3	8.1	10.8	17.9
	6	2.1	—	—	1.6	7.4	11.1	10.7
	7	1.9	1.2	0.7	1.6	5.8	9.3	15.5
	Mean	2.0	1.4	0.7	1.7	8.3	11.5	14.6
	CV	26%	18%	—	28%	20%	14%	36%
2A	1	5.1	—	—	4.7	20.1	30.0	31.8
	2	—	—	—	—	18.6	—	28.4
	3	5.0	—	—	4.1	21.7	30.7	28.2
	4	5.1	2.9	2.1	3.5	18.9	27.5	40.7
	5	3.2	—	—	4.3	22.3	29.8	37.6
	6	6.0	—	—	4.8	18.9	29.7	30.5
	7	3.8	3.1	0.7	5.5	14.9	24.2	39.6
	Mean	4.7	3.0	1.4	4.5	19.3	28.7	33.8
	CV	21%	4%	73%	15%	13%	9%	16%
3A	1	30.0	—	—	19.4	40.7	90.1	89.1
	2	—	—	—	—	39.1	—	89.8
	3	29.7	—	—	19.0	52.9	102.0	102.0
	4	25.2	10.5	14.7	19.4	57.2	102.0	113.0
	5	23.7	—	—	18.1	56.1	97.9	107.0
	6	28.2	—	—	19.5	43.9	90.7	95.1
	7	25.5	11.6	15.8	23.9	48.0	97.4	126.0
	Mean	27.0	11.1	15.3	19.9	48.3	96.6	103.0
	CV	10%	7%	5%	10%	15%	5%	13%

Abbreviations: MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; CV, coefficient of variation.

^aTotal inorganic As includes As^{+3} and As^{+5} .

^bSum of inorganic arsenic, MMA, and DMA.

^cTotal As determined by either atomic absorption or inductively coupled plasma-mass spectrometry.

^dResult is close to the method detection limit.

Table 8. Mean concentrations of arsenic species

Sample	Arsenic (µg/l)						
	Inorganic			MMA	DMA	Sum As ^b	Total As ^c
	Total inorganic As ^a	As ⁺³	As ⁺⁵				
1A Lab mean	1.6	1.2	0.5	1.2	6.2	9.0	11.7
Concentration spiked	3.0	1.5	1.5	1.5	4.0	8.5	8.5
Percent recovery	53%	80%	33%	81%	155%	106%	138%
2A Lab mean	4.3	2.8	1.2	4.0	17.3	25.6	30.9
Concentration spiked	6.0	4.0	2.0	6.0	16.0	28.0	28.0
Percent recovery	72%	70%	60%	67%	108%	91%	110%
3A Lab mean	26.6	10.9	15.1	19.4	46.3	92.3	100.0
Concentration spiked	25.0	12.5	12.5	22.0	44.0	91.0	91.0
Percent recovery	106%	87%	121%	88%	105%	101%	110%

Abbreviations: MMA, monomethylarsonic acid; DMA, dimethylarsinic acid. The results have been corrected for arsenic species in the unspiked urine.

^aTotal inorganic As includes As^{+3} and As^{+5} .

^bSum of inorganic arsenic, MMA, and DMA.

^cTotal As determined by either atomic absorption or inductively coupled plasma-mass spectrometry.

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